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Xanthomonas gardneri – Characterization and Resistance of Bulgarian Tomato Varieties

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Abstract

Xanthomonas gardneri and *X. vesicatoria* are the causal agents of bacterial spot of tomato in Bulgaria. *X. gardneri* was identified in the aria of our country on variety Bela for the first time in 2010. Symptoms on the leaves were dark, circular to irregular, water-soaked spots surrounded by chlorotic halos on fruit formed scabs. *X. vesicatoria* and *X. gardneri* can be identified through bacterial isolation only. *X. gardneri* was gram-negative, aerobic rods with a single flagellum. Bacterial colonies on peptone sucrose agar were yellow and rose with smooth margins. Starch and pectate hydrolysis tests were not positive. BIOLOG^{TM} GN2 (Biolog, Inc., Hayward, CA, USA) microplates were used for obtaining metabolic fingerprints. The metabolic profile of tomato isolates with bacterial spot symptoms was typical for *X. gardneri*. The distribution of *X. gardneri* on tomato crops around the country required a research on the resistance of the Bulgarian tomato varieties. Immune tomato varieties were not identified. Resistant genotypes (with mean score 0.01-0.60) were Nikolina F_1 - determinate large-fruit variety for mid-early field production and IZK Alya - variety of cherry type. Highly sensitive (with mean score over 3.0) were two determinate varieties - Bela and Venera, suitable for industrial processing.

Keywords: tomatoes, *Xanthomonas gardneri*, resistance, BIOLOG

Introductions

Bacterial spot of tomato plants has been observed in areas with high humidity and warm climate. The disease causes significant losses, estimated of about 10-20% per year and it is one of the economically most important diseases in all continents where *Solanum lycopersicum* L. is cultivated. The disease is caused by phenotypic and genotypic heterogeneous strains of the genus *Xanthomonas* (*X. euvesicatoria*, *X. gardneri*, *X. perforans* and *X. vesicatoria* (Jones et al., 2004).

Xanthomonas vesicatoria and *X. gardneri* are causing the bacterial spot of tomato in Bulgaria (Bogatzevska, 2002; Bogatzevska and Sotirova, 2002; Kizheva et al., 2013). *X. gardneri* was established for the first time in the country in 2010 on Bela variety in South region of Bulgaria (Kizheva et al., 2013; Stoyanova et al., 2014).

Novel disease symptoms on tomato fruits similar to "*bird eye*" was identified in 1957 in the former Republic of Yugoslavia by Sutitic, (1957) as *Pseudomonas gardneri*. Symptoms on the fruits are similar to the ones caused by *X. vesicatoria*, but they differ by the white oreol around the scabs. Bacterial pathogen is related to *X. vesicatoria* by biochemical and morphological characteristics: the colonies colour in intense yellow on the agarose media, the starch does not hydrolyze, and acids from dextrin and manitol are not extricated.

Later, Dye (1966) examined *P. gardneri* and determined that it was a typical xanthomonad according to morphological and biochemical tests. Dye (1966) compared bacterial specie with a large number xanthomonads using standard procedures and concluded that *P. gardneri* was a synonym of *X. vesicatoria*. Both bacteria produce typical for bacterial spot symptoms on tomato and could be distinguished by isolations only. This specie is not included in the genus *Xanthomonas,* because its taxonomic relation is not clear enough (Dye et al., 1980; Vauterin et al., 1995; Young et al., 1996; Jones et al., 1998). On the basis of fat acid analyses, absorption of number of carbon sources and sequencing analyses of 16S rRNA at the beginning of XXI century was proved that bacteria is phylogenetically related to the rest of the xanthomonads, causal agents of bacterial spot and it was placed into the group of D-*X.gardneri* (Jones et al., 2004).

X. gardneri is spread in Costa Rica (Bouzar et al., 1999), Canada and Brazil (Quezado-Duval et al., 2004), Russia (Kornev et al., 2009), the USA (Kim et al., 2010; Ma et al., 2011), and the Islands in the Indian Ocean (Hamza et al., 2010). In the world resistant samples of *X. gardneri* have not been established (Sim et al., 2012).

The lack of resistance to bacterial spot increases both the financial cost and environmental impact of tomato production while reducing yield and quality. Because several bacterial pathogens can be present in the same field, developing varieties with resistance to multiple diseases is a desirable goal.

The spread of *X. gardneri* in typical tomato production regions in the country on Bulgarian selection variety in recent years makes it necessary carry out research for establishing their reactions of resistance.

Materials and methods

Pathogen characteristic

Isolations

Bacteria were isolated by the method of the dilution (Rudolph et al., 1990) from tomatoes – leaves, flowers and fruit from Bulgarian varieties with symptoms of bacterial spot on KBM and JDC (Schaad et al., 2001). Samples were collected in mass seed producing crops in North Bulgaria (near Pavlikeni and Veliko Tarnovo), South Bulgaria (Plovdiv, and Haskovo), and Western Bulgaria (Sofia and Kostinbrod Bozhurishte) during 2012-2014.

Pathogenicity

The pathogenic properties of the isolates (24 strains) were tested by vacuum infiltration of tomato and pepper (Bogatzevska, 2002).

Identification

BIOLOG^{TM} GN2 (Biolog, Inc., Hayward, CA, USA) microplates were used for obtaining metabolic fingerprints. The bacteria were incubated on BUG agar at 28°C for 24h prior to analysis. The procedure was held according to the manufacturer's instructions. The results were cluster analyzed to differentiate the strains according to their metabolic profiles. Amylase activity was tested on starch agar medium (Schaad et al., 2001). The type cultures *X. vesicatoria* NBIMCC 2427, *X. euvesicatoria* NBIMCC 8731 and *X. gardneri* NBIMCC 8730 were used.

Table. 1. Differentiating properties of the Bulgarian strains - *X. vesicatoria* and *X. gardneri* according to the metabolic patterns

Substrates	*X. vesicatoria*	*X. gardneri*
Glycogen	v+	-
Tween40	v	v
Cellobiose	v	+
Gentibiose	v+	v-
α-D-lactose	-	v
Maltose	v	+
D-Mannitol	v	-
Turanose	v	-
cis-Aconitic acid	v	-
α-hydroxybutiric acid	v	-
α-kato butyric acid	v	-
Lactic acid	v	v-
Malonic acid	v	-
Propionic acid	v	-
Succinamic acid	v	v
D-alanine	v	v-
L-alanine	v	v-
L-alanyl-glycine	v	v-
Asparagine	v-	-
L-aspartic acid	v	v-
L-Glutamic acid	v+	v
Glycyl-L-Aspartic acid	v-	-
Glycyl-L-Glutamic acid	v	v-
Hydroxy L-Proline	v	v-
L-proline	v	-
L-Threonine	v	-
γ-aminobutyric acid	v	-
Urocanic acid	v	-
Inosine	v-	-
Uridine	v-	-
Butanediol	v	-
Glycerol	v	-
α-glycerol-phosphate	v	-
glucose-1-phosphate	v	-
glucose-6-phosphate	v	-

(+): positive; (-): negative; (v): variable, (v-): more than 75% negative; (v+): more than 75% positive

Resistance of Bulgarian tomato varieties

Plant material **-** total of 33 tomato varieties were included in industrial production in Bulgaria. They were distributed by direction of production - early and mid-early field production; type of growth habitus - indeterminate and determinate; average fruit weight - large-fruit varieties, small-fruit varieties used for the production of peeled tomatoes and cherry type; content of lycopene and β-carotene in fruits.

Bacterial pathogen – *X. gardneri* – 67t-PT

Inoculation procedure - plants of each variety (20 pieces or more) in growth stage of the 5-6th leaf were infected with bacterial suspension of 36h culture of *X. gardneri* in a concentration of 10^8 cfu/ml using the vacuum-infiltration method

(Bogatzevska, 2002). The infected plants of each variety were individually grown in a nutrient solution under a room temperature.

Evaluation of the disease – number of diagnostic lesions on the leaves from bacterial spot was registered 4-5 days after infiltration. Mean score (ms) of disease infestation was calculated on the scale of Sotirova and Beleva (1975). The classification of the varieties in groups was made on the grounds of the mean score (ms): immune - 0 (I); resistant: 0.01 – 0.6 (R); moderately sensitive: 0.61 – 1.49 (MS); sensitive: 1.50 – 2.99 (S); highly sensitive over 3 (SS).

Results and Discussion

Characterization of *X. gardneri*

The yellow bacteria isolated formed dark, circular to irregular, water-soaked spots surrounded by chlorotic halos on the tomato and pepper leaves when inoculated artificially.

Symptoms on the tomato plants recorded under natural infection and after artificial inoculation were typical for bacterial spot. Symptoms on the leaves were dark, circular to irregular, water-soaked spots surrounded by chlorotic halos; on fruits formed scabs. *X. vesicatoria* and *X. gardneri* can be identified through bacterial isolation only. Pathogenic bacteria isolated (24 strains) were identified as *X. gardneri* through BIOLOG^{TM}.

X. gardneri was gram-negative, aerobic rods with a single flagellum. Bacterial colonies on peptone sucrose agar were yellow and rose with smooth margins. Metabolic characteristic of 24 strains studied corresponded to type strain of *X. gardneri* NBIMCC 8731 as well as to the description of specie reported by Stoyanova et al. (2014). They hydrolyzed slightly or did not hydrolyzed starch, did not grow in medium with *cis*- aconitate, glycogen, D-mannitol, turanose, α-hydroxybutiric acid, α-keto butyric acid, proptonic acid, asparagines, glycil-L-aspartic acid, glycil-L-glumatic acid, L-proline, L-threonine, γ-aminobutyric acid, urocanic acid, inosine, uridine, butanediol, glycerol, α-glycerol-phosphate, glucose-1-phosphate, glucose-6-phosphate. They absorbed in 100% cellobiose and D-mannose. The Bulgarian strains did not use acetic acid, alaninamide, D-alanine, L-alanine, L-aspartic acid, and hydroxy L-proline (table 1). A notable characteristic of *X. gardneri* is its significantly lower nutritional properties. This specie generally utilizes 15 substrates less than *X. vesicatoria*. Since the reaction of *X. gardneri* to these carbon sources is always negative, this specie can easily be stinguished from the *X. vesicatoria* (Stoyanova et al., 2014).

Pathogenic population of causing agents of bacterial spot on Bulgarian tomato was heterogenic and consisted of two species: *X. vesicatoria* and *X. gardneri.* Bulgarian isolates of *X. gardneri* referred to pepper- tomato pathotype. Metabolic profiles of both pathogens were clearly distinguished. Automatic system BIOLOG^{TM} could be used for rapidly identification between both species.

Resistance of Bulgarian tomato varieties to *X. gardneri*

The tested Bulgarian tomato varieties differ by their degrees of resistance to bacterial spot, caused by *X. gardneri*. Varieties with immune reaction were not identified.

Varieties for early field production included in the study were susceptible, with having ms of 1.69 – 2.19 (table 2). Plants with hypersensitive reaction (HR) and highly sensitive reaction were not recorded. Highly sensitive reaction was recorded in v.Balkan $F_{1,}$ which is one of the oldest varieties – standard for industrial early field production. The rest of the recently developed varieties did not show significant improvement in their resistance to *X. gardneri*.

Variety IZK Alya of cherry type was the one resistant to *X. gardneri* out of the varieties with indeterminate habit for mid- early field production (table 3). That variety has resulted from the intraspecific hybridization with the wild type *Solanum racemigerum*.

Moderately sensitive reaction to *X. gardneri* was recorded in v.Plovdivska karotina, a result from long-term breeding program for developing tomato releases with enhanced content of natural antioxidant (β-carotene, lycopene and ascorbic acid) in the fruits. Wild type *Solanum chilense* was incorporated in the variety pedigree.

Varieties Rozalina rossa F_1 and Rozov blyan with pink ground colour of the fruits showed moderately sensitive reaction to *X. gardneri* as well as hybrid variety Naslada F_1. Highly sensitive reaction with rates 3 and 4 of the plants showed varieties Rila F_1 and Kalina $F_{1,}$ widely distributed in the country for field production.

Wide range of disease development was observed among determinate tomato varieties for mid-early filed production- from resistance in v.Nikolina F_1 (ms= 0.45) to highly sensitive in v. Venera (ms=3.43), (table 4). Moderately sensitive reaction showed Elena prima $F_{1,}$ Trapesitza, Stela among the large-fruited varieties; and Topaz, Kapri and Olimp $F_{1,}$ – among the varieties for industrial processing.

Table 2. Resistance of tomato varieties for early field production to *X. gardneri*

Variety	Plant number	*0/HR	1	2	3	4	ms
Balkan F_1	22	0	4	12	4	2	2.19
IZK Niki F_1	23	0	9	12	2	0	1.69
Riya F_1	22	0	6	15	1	0	1.77
Ragby F_1	24	0	7	13	4	0	1.88

* Sotirova and Beleva (1975) degree scale; HR: hypersensitivity reaction; ms: mean score

Table 3. Resistance of indetereminate tomato varieties for mid-early field production to *X. gardneri*.

Variety	Plant number	*0/HR	1	2	3	4	ms
Mid-early indeterminate varieties							
Rila F_1	23	0	6	15	1	1	1.87
Kalina F_1	23	0	7	14	2	0	1.78
Naslada F_1	20	0	13	7	0	0	1.35
Opal F_1	20	0	5	15	0	0	1.75
Rozalina rossa F_1	20	2	12	6	0	0	1.20
Mid-early indeterminate varieties – *cherry type*							
IZK Alya	20	9	11	0	0	0	0.55
Mid-early indeterminate varieties *with increased β-carotene content*							
Plovdivska karotina	21	1	11	5	0	0	1.00
Variety from local selection							
Rozov blyan	21	2	12	7	0	0	1.24
BG Fantazia	24	1	10	13	0	0	1.50

*Sotirova and Beleva (1975) degree scale; HR: hypersensitivity reaction; ms: mean score

Table 4. Resistance of the determinate tomato varieties for mild-early field production to *X. gardneri*

Variety	Plant number	*0/HR	1	2	3	4	ms
Mid-early determinate large fruit varieties							
Nikolina F_1	20	11	9	0	0	0	0.45
Elena primaF_1	20	3	9	8	0	0	1.25
Kopnezh F_1	20	0	2	15	3	0	2.05
Milyana	22	1	12	6	3	0	1.50
Bononia	21	0	0	7	8	6	2.96
Trapesitza	20	0	13	7	0	0	1.35
Merkuriy	20	0	4	10	6	0	2.10
Yana	20	0	8	11	1	0	1.65
Solaris	20	0	5	13	2	0	1.85
Stela	20	1	9	10	0	0	1.45
Topaz	22	2	15	5	0	0	1.14
Marti	20	0	8	11	1	0	1.65
Mid-early determinate varieties for industrial processing, of the type peeled tomatoes							
Bela	22	0	0	10	2	10	3.00
Venera	21	0	0	0	12	9	3.43
Zhaklin	24	0	8	16	0	0	1.67
Kapri	20	3	15	2	0	0	0.95
Vodolei F_1	24	0	12	12	0	0	1.50
Olimp F_1	28	0	14	14	0	0	1.05
Mid-early determinate varieties with increased content of β-carotene							
Karobeta	20	0	3	13	4	0	2.05
Neven	20	0	2	7	9	2	2.55

*Sotirova and Beleva (1975) degree scale; HR: hypersensitivity reaction; ms: mean score

Highly sensitive reaction was recorded only in the group of determinate varieties of peeled type - Bela and Venera.

Determinate varieties with increased -carotene content of the fruits were included in the experiment. Variety Karobeta possess large fruits (120-160 g), oblate to circular shape in longitudinal section and v.Neven - suitable for industrial processing, with having fruits with elongated-cylindrical shape and average weight 55-70 g. Variety Karobeta and v. Neven have been developed by hybridization with wild type *S. pimpinelifolium ƒ galapagon* and *S. hirsutum* respectively. The expected higher degree of resistance *to X. gardneri* in those genotypes that were developed through interspecific hybridization was not confirmed in our study.

Results from the study reviled that there is no immune varieties, but there were ones with moderate sensitiveness. Varieties IZK Alya, Plovdivska karotina, Rozalina rossa F_1, Rozov blyan, BG Fantazia, Nikolina F_1, Elena prima F_1, Milyana, Stela, Topaz and Kapri showed HR reaction to the pathogen, which defined them valuable for the future breeding work.

Conclusions

Pathogenic population of bacterial spot on Bulgarian tomato varieties included in the study was heterogenic, involving two species: *X. vesicatoria* and *X. gardneri.* Metabolic profiles of *X. vesicatoria* and *X. gardneri* were clearly distinguished. Automatic system BIOLOG^{TM} GN2 could be used for fast and reliable identification between both species. Immune tomato varieties from Bulgaria were not established. Resistant reactions to bacterial spot, caused by *X. gardneri* were recorded in v. Nikolina F_1 (determinate, large-fruited) and v. IZK Alya (indeterminate, cherry type, a result from interspecific hybridization with wild type *Solanum racemigerum*). Moderately sensitive to the pathogen were varieties Plovdivska karotina, Rozov blyan and Rosalina rossa F_1 and determinate varieties Elena prima F_1, Trapesitza, Stela, Topaz, Olimp $F_1$and Kapri. Highly sensitive to *X. gardneri* were varieties Bela and Venera of determinate growth habit, oval-shaped fruits and suitable for industrial processing.

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